Tissue-Specific Expression of the Mouse Dioxin-Inducible P_1450 and P_3450 Genes: Differential Transcriptional Activation and mRNA Stability in Liver and Extrahepatic Tissues

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Expression of the P_1450 and P_3450 genes was examined in liver and five extrahepatic tissues of mice after they were treated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) or 3-methylcholanthrene. All six tissues were shown to have increased P_1450 and P_3450 mRNA concentrations after treatment with these inducers. P_3450 mRNA induction was more sensitive than P_1450 mRNA induction to small doses of TCDD in liver, kidney, and lung. When transcription run-on assays were compared with mRNA prevalence, control P_3450 mRNA in liver, kidney, and lung was shown to be 20 to 30 times more stable than control P_1450 mRNA. After TCDD treatment the increases in mRNA concentrations did not necessarily parallel the increases in transcriptional rate. Thus, the inducer appeared to enhance mRNA stability in some instances. This was evident for liver P_1450 mRNA, in which an 8-fold rise in transcription was associated with a 27-fold increase in mRNA content, and for kidney P_3450 mRNA, in which a 2-fold rise in transcription was accompanied by a 12-fold increase in mRNA content. In the kidney and lung of control and TCDD-treated mice, transcriptional rates of the P_3450 gene were at least 10-fold less than those of the P_1450 gene. These data indicate that even though both genes are controlled by the same receptor, striking tissue-specific differences in transcription and mRNA stabilization affect the final mRNA concentrations.

Among laboratory animals such as mice, rats, and rabbits, the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)-inducible P450 gene family comprises two genes: P_1450 and P_3450 in the C57BL/6N mouse (13); P450c and P450d, respectively, in the rat (38, 39); and form 6 and form 4, respectively, in the rabbit (18, 32). The P_1450 and P_3450 genes appear to lie adjacent to one another near the *Mpi-1* locus of mouse chromosome 9 (19), have most likely arisen via gene duplication at least 65 million years ago (24), and are regulated by the aromatic hydrocarbon (Ah) receptor, the gene product of the *Ah* locus (for a review, see reference 9).

Mouse P_1450 protein is known to metabolize benzo[a]pyrene better than P_3450 protein (31). Similarly, it has been reported that rat P450c protein metabolizes benzo[a]pyrene at least 50 times better than rat P450d protein (35). On the other hand, the rat P450d protein is more important than the rat P450c protein in the metabolism of 2-aminofluorene, 4-aminobiphenyl, and two promutagenic pyrolysis chemicals to active mutagenic and carcinogenic intermediates (23). Metabolism of benzo[a]pyrene and more than a dozen other polycyclic hydrocarbons is a well-established prerequisite to chemically induced malignancies in various laboratory animals (7, 12, 22, 34). Hepatic P_3450 mRNA and P_3450 protein are known to be several times more abundant than P_1450 mRNA (15) and P_1450 protein (30), respectively. Differences in chemical carcinogenesis, mutagenesis, drug toxicity, and teratogenesis have been highly correlated with allelic differences at the *Ah* locus (for reviews, see references 27 and 28). Because of an Ah receptor defect in homozygous recessive siblings of the (C57BL/6N)(DBA/2N)F_1 × DBA/2N backcross, foreign chemicals such as benzo[a]pyrene can effect dramatic differences in the P_1450 induction or P_3450 induction response,

metabolism, and pharmacokinetics. The resultant malignancies or toxicity thus reflect allelic differences at the *Ah* locus and are highly dependent on target tissue and the dosage and route of administration of the test compound (27). In each tissue the amount of procarcinogen metabolism that can be attributed to the P_1450 protein or to the P_3450 protein therefore may be important in understanding dissimilarities in tumorigenesis and drug toxicity that are known to be associated with allelic differences at the *Ah* locus.

In this study, P_1450 and P_3450 mRNA levels were quantitated in mouse liver and five extrahepatic tissues as a function of dose and time after intraperitoneal treatment with TCDD. Nuclear transcription run-on experiments in liver, kidney, and lung were also performed. Striking differences in tissue-specific transcriptional activation and transcript stability were found.

MATERIALS AND METHODS

Animals. C57BL/6N mice (male, sexually mature) were obtained from the Veterinary Resources Branch, National Institutes of Health (Bethesda, Md.). Unless otherwise indicated, groups of 10 mice received intraperitoneal TCDD (100 μg/kg) or 3-methylcholanthrene (200 mg/kg) and were killed at various intervals up to 24 h. Liver, kidney, spleen, lung, large intestine, and small intestine were removed, put on ice, and prepared for mRNA quantitation or nuclear transcription run-on experiments. All experiments were performed a minimum of three times to ensure reproducibility.

mRNA quantitation. Poly(A)-enriched or total RNA from the six tissues was prepared by standard methods (5) and loaded onto a minifold II slot-blot apparatus (Schleicher & Schuell, Inc., Keene, N.H.) by the protocol recommended by the manufacturer. Poly(A)-enriched RNA (1 μg) or total RNA (15 μg) was immobilized in each slot and hybridized

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with each of three nick-translated probes: pP_1450-3', the 3'-specific portion (1,259 base pairs) of the P_1450 cDNA (24); pP_3450-3', the 3'-specific portion (1,304 base pairs) of the P_3450 cDNA (24); and pAC.H8-H, a human cardiac actin gene (17) that was a gift of Narayana Battula (National Cancer Institute, Bethesda, Md.). The conditions of prehybridization, hybridization, and posthybridization washing of the slot-blot membranes were performed as described previously (6). Relative P_1450 and P_3450 mRNA concentrations were determined by the densitometric ratio of P_1450 mRNA/actin mRNA and P_3450 mRNA/actin mRNA, respectively, on x-ray films that had been exposed to the slot-blot filters. The actin mRNA levels served to normalize the amount of RNA present in each slot and was previously determined not to be affected by TCDD or 3-methylcholanthrene. In some experiments the radioactivity of the cDNA bound to the paper was directly determined by scintillation counting, and these values correlated very well with the numbers obtained by direct densitometric readings of the autoradiographs.

Transcription with nuclei in vitro. Purified nuclei (44) were isolated from liver, kidney, and lung. The in vitro nuclear transcription assays were performed essentially as described previously (26). Newly transcribed RNA was subjected to slot-blot hybridization with the pP_1450-3', pP_3450-3', and actin probes. The densitometric ratio of P_1450 transcripts/actin transcripts and P_3450 transcripts/actin transcripts was found to be highly reproducible and more sensitive, especially in nonhepatic tissues which have a very low basal rate of transcription, than the previously described filter hybridization technique (15).

RESULTS

Differential sensitivity to inducer. P_3450 mRNA induction was more sensitive than P_1450 mRNA induction to lower doses of TCDD in liver, kidney, and lung (Fig. 1). The reverse was seen in spleen, however, and no differences in differential sensitivity to TCDD were found for the two genes in the large or the small intestine. This differential sensitivity to induced mRNA accumulation has been previously reported for the expression of these two genes in liver (20, 41).

Treatment of the mice with 3-methylcholanthrene, at a dose (200 mg/kg) more than 10 times greater than the 50% effective dose for 3-methylcholanthrene (2), produced in all six tissues a pattern of P_1450 and P_3450 mRNA induction that was quite different from that induced by a high dose of TCDD (Fig. 1). Different rates of uptake and distribution (pharmacokinetics) of 3-methylcholanthrene, as compared with those of TCDD, may explain the marked dissimilarities in patterns of P_1450 and P_3450 mRNA induction. Alternatively, there might exist different properties in the Ah receptor (9) among these six tissues.

Induction kinetics of mRNA. In nontreated animals (Fig. 2A and B), the hepatic basal P_3450 mRNA level was at least 25 times greater than the hepatic basal P_1450 mRNA level. In fact, the liver constitutive P_3450 mRNA level was about the same as the maximally induced liver P_1450 mRNA level. In contrast, for any of the five extrahepatic tissues examined, the control P_3450 mRNA level was no more than three times greater than the control P_1450 mRNA level.

In liver and kidney (Fig. 2A and B), the maximally induced P_3450 mRNA concentration was at least fivefold higher than the maximally induced P_1450 mRNA concentration. No noteworthy differences were observed in spleen. P_1450 mRNA was somewhat more inducible than P_3450 mRNA in

lung, large intestine, and small intestine. TCDD-induced increases in both mRNAs in all six tissues were reproducibly detectable.

Transcriptional activation. Increases in mRNA concentrations can be the result of de novo synthesis, mRNA stabilization, or some combination of the two. For mouse liver (15), it is known that TCDD and 3-methylcholanthrene primarily evoke increases in P_1450 and P_3450 mRNA by way of transcriptional activation of both genes. By means of hybridization and slot-blot autoradiographic analysis of nuclear run-on transcripts (Fig. 3), the rate of transcriptional activation in liver was found to be similar for the P_1450 and P_3450 genes. These results are consistent with results of a previous study (15). In contrast, in the kidney and lung the basal transcriptional rate of P_3450 was less than one-tenth that of P_1450, yet reproducible TCDD-induced increases in P_1450 and P_3450 transcriptional activation occurred in both tissues (Fig. 3).

Comparison of transcriptional rate and mRNA prevalence for the two genes. The transcription and mRNA data in control and maximally induced liver, kidney, and lung are compared in Table 1. In control liver the transcriptional rates of P_1450 and P_3450 were similar, yet P_3450 mRNA was 25 times more prevalent than P_1450 mRNA. This observation suggests a 25-fold difference in mRNA stability between hepatic basal P_1450 and P_3450. In TCDD-induced liver the transcriptional rates of P_1450 and P_3450 both rose similarly (about 8-fold), whereas P_1450 mRNA rose about 25-fold and P_3450 mRNA rose 6-fold. Thus, instead of a 25-fold difference in the ratio of P_1450/P_3450 mRNA prevalence, there was about a 6-fold difference after TCDD treatment. These data indicate that TCDD caused a three- to fourfold enhancement in liver P_1450 mRNA stability.

In control kidney the transcriptional rate of P_1450 was 10 times greater than that of P_3450, while P_3450 mRNA was three times more prevalent than P_1450 mRNA. This finding suggests that kidney-uninduced P_3450 mRNA is 30 times more stable than kidney-uninduced P_1450 mRNA. Following TCDD treatment the kidney transcriptional rates of both genes increased about the same (2- to 3-fold), so that P_1450 transcription remained 10- to 20-fold greater than P_3450 transcription. The P_1450 mRNA increase paralleled the P_1450 transcriptional rate increase, whereas the P_3450 mRNA increase (12-fold) did not parallel the P_3450 transcriptional rate increase (2-fold). These data indicate that TCDD causes about a sixfold enhancement in P_3450 mRNA stabilization. The other major difference between the two genes in the kidney is the striking absence of significant P_3450 gene transcription.

In control lung the transcriptional rate of P_1450 was 10 times greater than that of P_3450, while the P_3450 mRNA was twice as prevalent as P_1450 mRNA. This observation suggests a 20-fold difference in mRNA stability between lung basal P_1450 and P_3450. In TCDD-induced lung the transcriptional rates of both genes increased about the same (three- to fourfold), and the TCDD-induced mRNA levels were also similar. These data indicate that P_3450 mRNA stability remains about the same in control and TCDD-induced lung. The major difference between the two genes in the lung was that, similar to what was found in kidney, there was very little P_3450 gene transcription.

DISCUSSION

In this study we have shown direct evidence for TCDD induction of both P_1450 and P_3450 mRNA in C57BL/6N mouse liver, kidney, spleen, lung, large intestine, and small
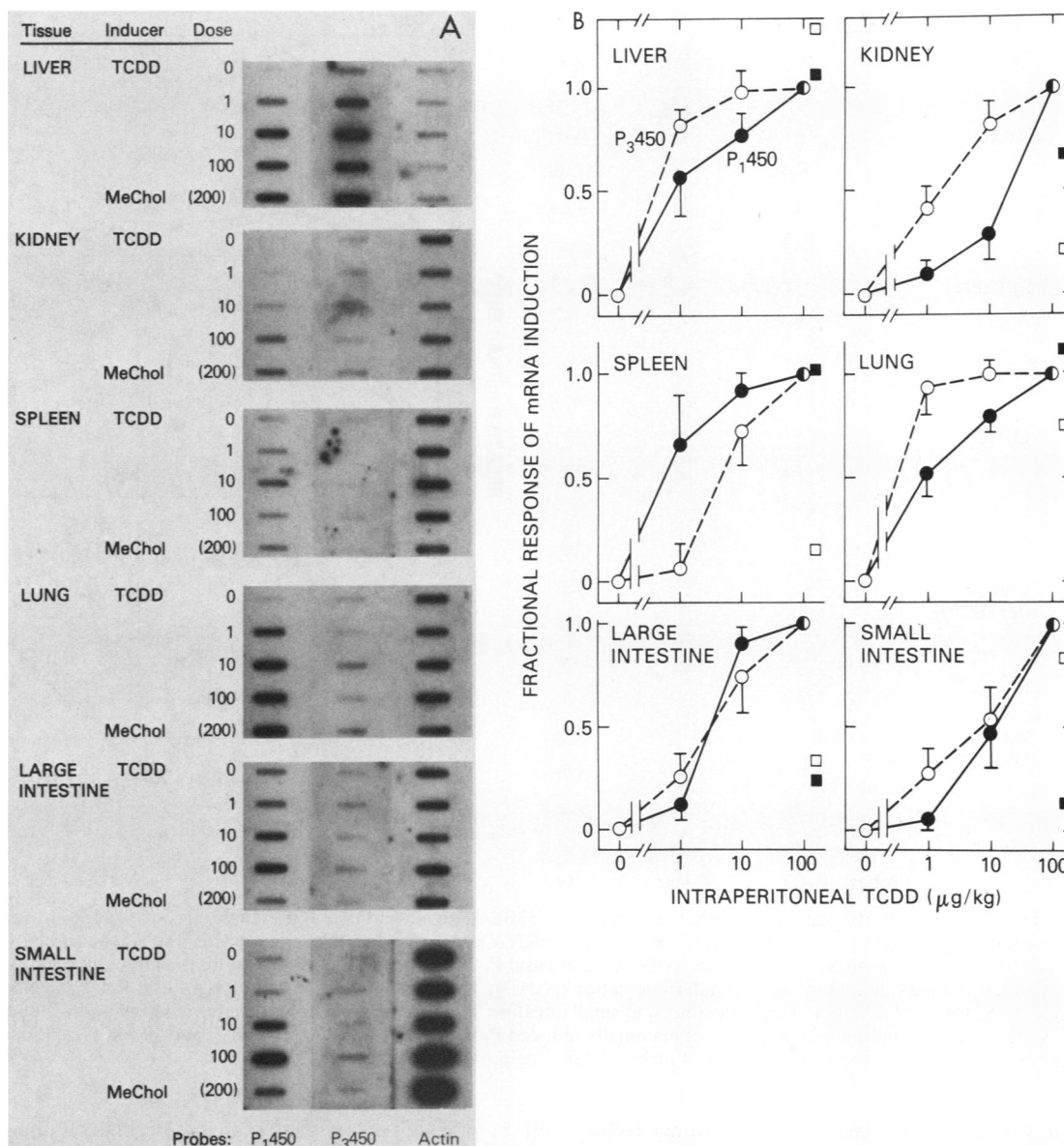
FIG. 1. Differential sensitivity of $P_1 450$ and $P_3 450$ mRNA induction to TCDD dosage. (A) Representative slot blot. Fourteen hours after the various doses of TCDD (in micrograms per kilogram) or the 200-mg/kg dose of 3-methylcholanthrene (MeChol) were given, total RNA was prepared from the six tissues, and 15 µg of total RNA was immobilized in each slot and hybridized to each of the three nick-translated probes. (B) Fractional response of $P_1 450$ and $P_3 450$ mRNA induction as a function of TCDD concentration. Fractional response curves as a function of TCDD concentration allow one to estimate curve symmetry by probit analysis and a median effective dose for 50% of the maximal induction response. Constitutive levels are regarded as zero, and the maximally induced levels are regarded as 1.0 (11). The level of $P_1 450$ (■) and $P_3 450$ (□) mRNA induction by 3-methylcholanthrene (200 mg/kg), relative to the $P_1 450$ and $P_3 450$ mRNA levels induced maximally by TCDD (100 µg/kg), are shown. Circles and bars denote mean ± standard deviation for three different experiments. At the highest TCDD dose for 14 h, the fold induction of $P_1 450$ mRNA was 26, 3, 2, 8, 6, and 13; and the fold induction of $P_3 450$ mRNA was 5, 6, 4, 2, 2, and 3 for liver, kidney, spleen, lung, large intestine, and small intestine, respectively. Further details are provided in the text.

intestine. Results of nuclear transcription run-on experiments in liver, kidney, and lung demonstrated large differences in mRNA prevalence relative to the rates of transcriptional activation. Interpretation of the transcription run-on assay requires acceptance of a tacit assumption that this method is a valid measure of changes in relative rates of transcription. This type of transcription run-on experiment has been shown to be principally due to elongation of preinitiated chains (8, 16, 42). Therefore, in this study we assumed that there is little transcription reinitiation and little

alteration in the relative rates of chain extension between the two genes in the transcription assay. Unfortunately, transcriptional rates for the $P_1 450$ and $P_3 450$ genes cannot be measured in the intact animal. This is due to the large nucleotide pool size and the fact that these genes encode low-abundance mRNAs. The $P_1 450$ and $P_3 450$ transcripts therefore cannot be adequately radiolabeled, relative to total RNA content and total transcription. Mouse cell cultures would be more feasible for attempting such direct in vivo labeling, but the established line that has been well charac-
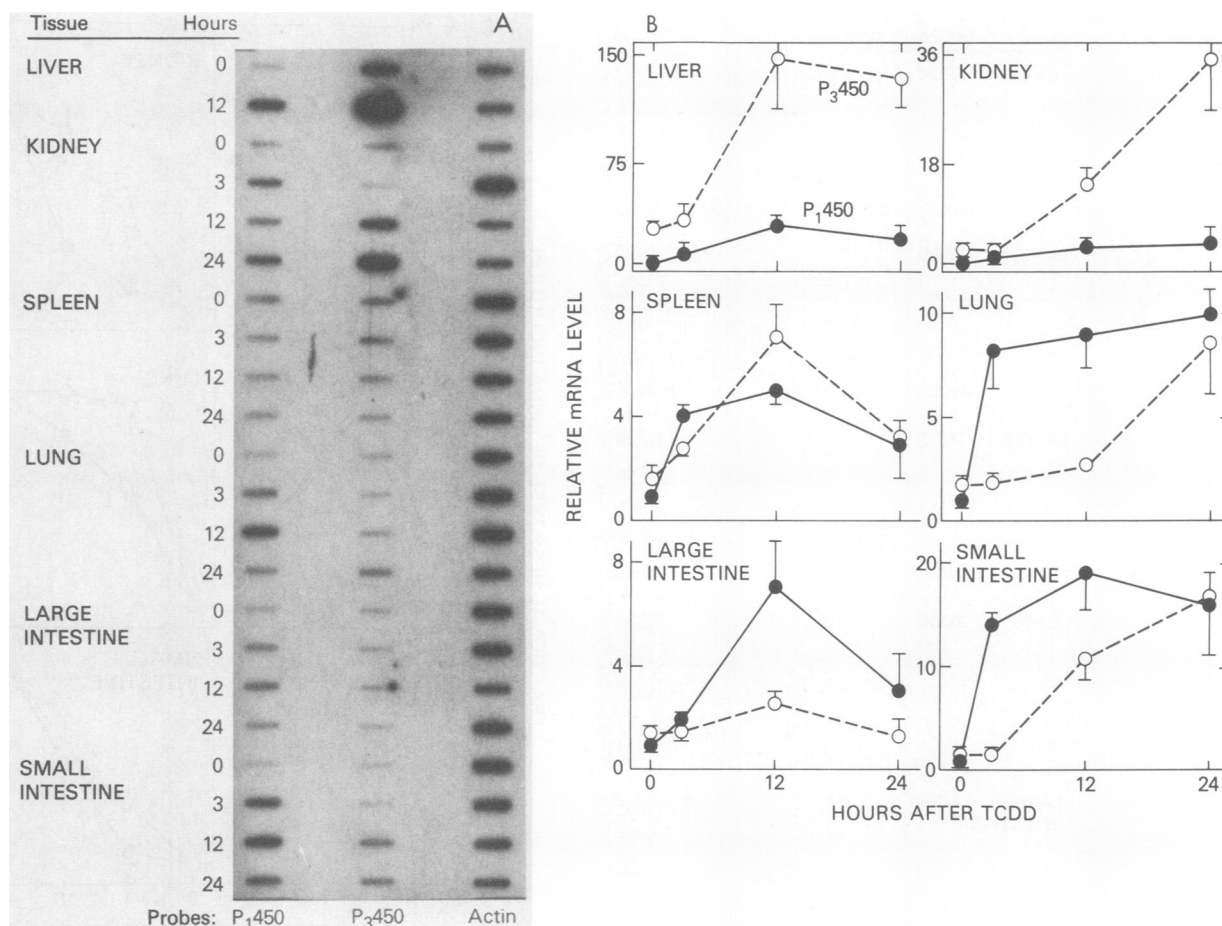
FIG. 2. Time course of P_1450 and P_3450 mRNA induction in C57BL/6N liver and five extrahepatic tissues. (A) Representative slot blot. Each slot contains 1 µg of poly(A)-enriched RNA. (B) Relative mRNA levels as a function of time after the single intraperitoneal dose (100 µg/kg) of TCDD. One unit of mRNA corresponds to the level of basal P_1450 mRNA in that tissue. Note the large differences in values on the ordinates. Circles and bars denote mean ± standard deviation ($N = 3$). The ratio of basal P_3450 mRNA/basal P_1450 mRNA was 25, 3, 2, 2, 1, and 2 in liver, kidney, spleen, lung, large intestine, and small intestine, respectively. The fold induction of maximally induced P_3450 mRNA was 6, 12, 4, 4, 2, and 10; and the fold induction of maximally induced P_1450 mRNA was 27, 3, 5, 10, 7, and 19 for liver, kidney, spleen, lung, large intestine, and small intestine, respectively. Further details are provided in the text.

terized in this laboratory (the mouse hepatoma Hepa-1 cell line and variant lines [14]) exhibits P_1450 but not P_3450 gene expression (21).

Taken together, the data in Fig. 2 and 3 and Table 1, in combination with the implicit assumptions stated above, indicate remarkable differences in the expression of these two homologous genes under control by the Ah receptor. (i) Detectable increases in TCDD-induced transcriptional rates and mRNA concentrations were always found in all tissues examined. (ii) In control liver, kidney, and lung P_3450 mRNA was 20 to 30 times more stable than P_1450 mRNA. (iii) Compared with control P_1450 mRNA, TCDD-induced P_1450 mRNA was three- to fourfold more stable in liver. (iv) In kidney TCDD-induced P_3450 expression was primarily the result of an increase in P_3450 mRNA stability.

It has been well documented that inducible P_1450 but not P_3450 expression appears earlier in development (20, 29, 41), persists in cultured cells long after P_3450 and other forms of P450 are lost (1, 25, 36, 37, 40), and remains in relatively dedifferentiated tumors (33) and preneoplastic nodules (3) after P_3450 and other forms of P450 are no longer expressed. The P_1450 gene is constitutively expressed and inducible in mouse hepatoma Hepa-1 cultures, whereas P_3450 gene ex-

pression is completely absent in this cell line (21). The same observation with the equivalent rat genes P450c and P450d was recently reported for primary cultures of adult rat hepatocytes (40). P_1450 gene expression, as measured by inducible benzo[a]pyrene metabolism, appears to be from a very primitive gene because it is known to exist in simple eucaryotes such as fungi (4, 10, 43). To our knowledge P_3450 gene expression, as measured by TCDD-inducible acetanilide 4-hydroxylase or estrogen 2-hydroxylase activity (41), has not been analyzed in these simple fungi. P_1450 thus appears to be correlated with more primitive and dedifferentiated conditions, whereas P_3450 may be associated with more differentiated functions. If these two genes arose by duplication more than 65 million years ago (24), it is conceivable that apparently diverse regulatory behavior has evolved despite some sort of common control in certain tissues by the TCDD-receptor complex.

Distinct differences between P_1450 and P_3450 in tissue-specific differential sensitivity to inducer were also found in this study (Fig. 1). The fact that P_3450 mRNA accumulation predominates over P_1450 mRNA accumulation at low TCDD doses (Fig. 1) is not simply related to differences in transcript stability between these two genes, because this differential
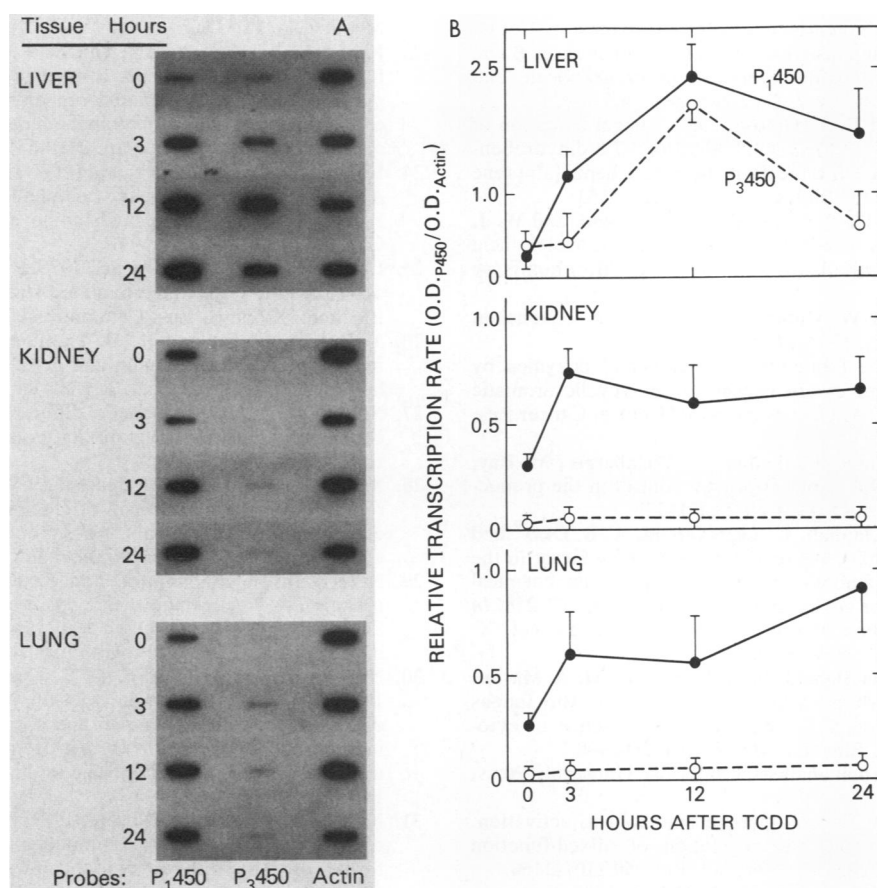
FIG. 3. Kinetics of the rate of transcriptional activation by TCDD of the liver, kidney, and lung P_1450 and P_3450 genes. (A) Representative slot blot. Each slot contains 9 μg of the pP_1450-3', pP_3450-3', or actin probe, as described in the text. (B) Relative transcriptional rate of the two genes as a function of time after the single intraperitoneal dose (100 μg/kg) of TCDD. The relative transcription rate was determined by the ratio of filter-bound P_1450 or P_3450 gene transcripts to filter-bound actin gene transcripts in the nuclear transcription run-on in vitro assay (26). OD, Optical density. Circles and bars denote mean ± standard deviation for three separate experiments. In each experiment nuclei were isolated from TCDD-treated mice at the designated times and subjected to transcriptional analysis.

sensitivity to inducer is observed in liver, kidney, and lung—despite the demonstration that the TCDD-induced P_3450 mRNA stabilization occurs in kidney but not in liver or lung (Table 1). Hence, each tissue appears to exhibit a specificity

by which these two genes, presumably lying in tandem and controlled by the same receptor (9, 19), are expressed in response to various TCDD doses. It will be interesting to characterize important nucleotide sequences in and near these two genes (13, 14) and the DNA-binding regulatory factors in these tissues, which must act together to bring about such striking differences in tissue-specific differential expression of the P_1450 and P_3450 genes.

TABLE 1. Relative transcription rates and mRNA prevalence in three tissues of control mice and after maximal induction by TCDD[a]

Tissue	Experimental condition	P_1450		P_3450	
		Transcription rate	mRNA prevalence	Transcription rate	mRNA prevalence
Liver	Control	1 ± 0.2	1 ± 0.3	1 ± 0.2	25 ± 5
	TCDD	8 ± 2	27 ± 6	7 ± 1	150 ± 30
Kidney	Control	1 ± 0.2	1 ± 0.3	0.1 ± 0.1	3 ± 0.8
	TCDD	3 ± 0.6	3 ± 1	0.2 ± 0.2	36 ± 6
Lung	Control	1 ± 0.3	1 ± 0.4	0.1 ± 0.1	2 ± 0.6
	TCDD	4 ± 0.9	10 ± 2	0.3 ± 0.2	8 ± 3

[a] Control and maximally induced values (means ± standard deviations; three experiments) have been extracted from Fig. 2 and 3 and are listed here. Control P_1450 transcriptional rates and mRNA levels in each tissue are arbitrarily assigned a value of 1.0 to compare more easily the differences between control P_1450 and control P_3450 transcriptional rates and mRNA levels and to view the changes that occur after TCDD treatment.

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